Hemicalcin (HCa), are highly basic 33-mer peptide toxins that fold along an inhibitor cysteine knot (ICK) motif, and whose pharmacological activity evokes activation of ryanodine receptors (RyR). We previously described $IpTx_a$, the first calcin toxin known to activate RyR, and demonstrated its ability to traverse cell membranes and alter Ca^{2+} release in intact cardiomyocytes. We introduce Hadrucalcin (HdCa), a new calcin toxin isolated from the venom of *Hadrurus gertschi*, a scorpion endemic to Guerrero, Mexico. Like other calcin toxins, HdCa is an amphipathic molecule with a stretch of positively-charged residues resembling the protein translocation domain of some cell-penetrating peptides. HdCa is distinguished from previously described congeners of the calcin family by two additional amino acids in its primary sequence. In the present study, we show that HdCa is capable of enhancing Ca^{2+} release from the sarcoplasmic reticulum (SR) of intact cardiomyocytes. Perfusion of field-stimulated cardiomyocytes with HdCa elicits three discernable effects:

1. up to 105% increase in fractional Ca^{2+} release compared to control;
2. up to 109% increase in Ca^{2+} transient amplitude compared to control, followed by a decrease to a new steady state as low as 63% of control; and
3. spontaneous Ca^{2+} release.

Significantly, HdCa perfusion of resting cardiomyocytes elicits discharge of SR Ca^{2+} stores and trains of trigger activity, both novel effects not previously observed with calcin toxins. Our results suggest that HdCa is a cell-penetrator and a powerful activator of RyRs, which has exciting translational potential for targeted delivery of drugs to RyR as novel therapeutic intervention in arrhythmogenic disease.

Platform BB: Excitation-Contraction Coupling

2632-Plat Structural and Functional Characterization of Ryanodine Receptor-Natrin Toxin Interaction

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Cysteine-rich secretory proteins (CRISPs) are found in many sources, especially in the mammalian reproductive tract and in salivary glands from reptile venoms. Some CRISPs can inhibit ion channels, such as the cyclic nucleotide-gated ion channel, potassium- channel, and calcium-channel. Natrin is a member of CRISPs that has been purified from snake venom, and its targets including the calcium-activated potassium channel and the calcium release channel/ryanodine receptor (RyR). Immuno-precipitation experiments showed that natrin binds specifically to type 1 RyR (RyR1) from skeletal muscle, inhibits the binding of [^{3}H]-ryanodine to RyR1, and blocks calcium release. Cryo-electron microscopy and single-particle image reconstruction analysis revealed that natrin binds to the clamp domains of RyR1. Further analysis showed that the binding of natrin altered the conformation of RyR1, and that binding of natrin to the four subunits of the RyR1 tetramer exhibited a positive cooperativity. Docking the crystal structure of natrin into the cryo-EM density map of RyR1+natrin complex indicated that the cysteine-rich domain of natrin is crucial for the binding. These findings reveal how natrin toxin blocks the RyR, and suggest a common interaction mode between CRISPs and RyR1 calcium release channel.

2633-Plat Dynamic FRET Signals between DPA and the α1s and β1a Subunits of the DHPR of Mammalian Skeletal Muscle Fibers

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Fluorescence resonance energy transfer (FRET) is a useful tool to investigate proximity between donor fluorescent molecules and Foster-energy-transfer acceptors. We took advantage of the rapid (τ~0.6 ms) voltage-dependent translocation of the non-fluorescent lipophilic anion dipicrylamine (DPA) across the transverse tubular system (TTS) membranes to investigate this molecule's FRET interaction with specific domains of the α1s and β1a subunits of the dihydropyridine receptor (DHPR). To this end, plasmids encoding for N- tagged EGFP α1s-DHPR (EGFP-α1s-DHPR) and N- or C-tagged ECFP β1a-DHPR were transfected into adult FDB muscles by *in vivo* electroporation. Two-photon microscopy demonstrated that N-tagged α1s-DHPR (EGFP-α1s-DHPR) was targeted to the surface and TTS membranes of the muscle fibers. In the absence of DPA voltage-clamped enzymatically-dissociated fibers expressing EGFP-α1s-DHPR did not show voltage-dependent optical transients. Staining with 5 μM DPA allowed the recording of signals whose sign and amplitude were consistent with the voltage-dependent translocation of DPA molecules across the surface and TTS membranes and their subsequent FRET interaction with the N-terminal of the α1s-DHPR (to within ~6–7 nm). Furthermore, the kinetic features of these FRET signals suggest that, in response to step depolarizations, the N-domain of α1s-DHPR possibly moves away from the internal membrane plane within ~20 ms after the onset of the pulse. In contrast with what was observed with the α1s-DHPR, the expression of fluorescently tagged β-DHPR failed to report voltage-dependent FRET signals, which suggests that this subunit may be located relatively far away with respect to the membrane plane.

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2634-Plat Titin Kinase (TK) Region Modulates Excitation-Contraction-Coupling In Skeletal Muscle

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It has been previously reported that in *cardiac* muscle the TK region regulates contractile function through effects on calcium handling. The role of the TK region in *skeletal* muscle contraction is unknown and here we investigated the effect of deletion of the TK region on skeletal muscle contractility. We used a conditional TK knock-out mouse model that has been described before. We determined titin isoform expression in LV, soleus, EDL, and FDB muscle and measured calcium transients in isolated LV myocytes using Fura2-AM, and in enzymatically dissociated FDB fibers using Fura2FF-AM. In skinned soleus fibers calcium sensitivity of force generation was determined as well as maximum Ca^{2+}-activated force. We found 40–50% truncated (kinase deficient) titin in LV, soleus, and EDL and 10–20% in FDB. Cardiomyocytes had a reduced rate of calcium reuptake as has been shown before in a cardiac-specific TK KO model. Surprisingly, in FDB fibers from KO mice calcium reuptake was faster compared to wt mice (tau: 7.8 vs 11.2 ms, KO vs wt respectively). Calcium sensitivity of force generation was reduced in skinned soleus muscle from KO (pCa50: 5.97 vs 6.11, KO vs wt respectively). Finally, maximum calcium-activated force was not significantly different between KO and wt soleus muscle (105 vs 96 mN/mm2, KO vs wt respectively). This work further establishes that the TK region modulates calcium kinetics. Interestingly we found that the effect is differential in cardiac and skeletal muscle (reduced rate of uptake in cardiac muscle and increased rate in skeletal muscle). Furthermore, the TK regulates calcium sensitivity of force generation in skeletal muscle. The TK-binding protein MURF-1 might be involved, as MURF-1 is known to degrade troponins and decreased binding capacity in the KO muscle might increase its cytosolic concentration and proteolytic activity.

2635-Plat Mechanical Alternans In Tric-a Deficient Skeletal Muscle Induced By Fatigue Stimulation

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The sarcoplasmic reticulum (SR) membrane of skeletal muscle contains potassium, chloride or proton channels to support charge neutralization during calcium release. Our recent studies have identified trimeric intracellular cation (TRIC) channels in the SR membrane as an essential permeability pathway for counter-ion movement associated with rapid Ca release from intracellular stores (Nature **448,** 78–82). TRIC has two isoforms, TRIC-A and TRIC-B, with TRIC-A highly expressed in striated muscle and TRIC-B ubiquitously expressed in all tissues. To further characterize the role of TRIC-A in excitation-contraction (E-C) coupling of skeletal muscle, we further examined TRIC-A deficient skeletal muscle. Based on biochemical assays, we found that the molar ratio of TRIC-A over ryanodine receptor (RyR1) is ~5:1 in rabbit skeletal muscle. Using SR vesicles isolated from the TRIC-A(−/−) mouse skeletal muscle, we observed no significant changes in basal SR membrane permeability to Ca ions, whereas significant defect in potassium permeability was observed in response to Ca release from the SR membrane. Although TRIC-A deficient skeletal muscle displayed similar force-frequency relationship and twitch contraction to the wild type (wt) muscle, the mutant muscle behaved distinctly from the wt muscle under stress conditions. In particular, ~50% of the TRIC-A(−/−) soleus muscle bundles exhibited "alternan" behaviors upon high frequency fatigue stimulation, i. e., transient, drastic increases in contraction, were observed to intersperse within the decreasing fatiguing force profile. These apparent aternan behaviors could be enhanced by addition of caffeine or SN-6, an inhibitor of the Na-Ca exchanger. The absence of TRIC-A may lead to instability in SR Ca handling properties and thus be a contributing factor to the observed alternan behavior or TRIC-A could directly interact with the Ca signaling machinery in skeletal muscle to maintain he stability and function of E-C coupling in muscle physiology.

2636-Plat Visualization of the 3-D Distribution of Proteins Involved in Cardiac Excitation-contraction Coupling Using a Novel Imaging Protocol

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Ca^{2+}-induced release of Ca^{2+} from the sarcoplasmic reticulum triggered by voltage-dependent trans-sarcolemmal Ca^{2+} fluxes is thought to form the basis of excitation-contraction coupling in cardiac myocytes. The sarcolemmal membrane architecture is complex including caveolar invaginations and a dense network of t-tubules near each z-disk. Such complexity in the organization of membrane structures is only partially resolved when imaged by confocal microscopy. The resolution can be effectively improved using a recently demonstrated technique (Chen-Izu et al. Biophys J. *91*:1–13, 2006; Soeller et al. PNAS *104*:14958–14963, 2007) to image single cells oriented vertically on the microscope stage. We have used this approach to visualize the distribution of the Na-Ca exchanger (NCX), Caveolin-3 (Cav3, a marker for caveolar membrane invaginations) and RyRs in enzymatically isolated and fixed rat myocytes at high resolution. While NCX labelling generally followed the geometry of the t-system (as outlined by Cav3 labelling), more intense labelling of the exchanger was observed at regularly spaced domains along z-line-associated t-tubules. Analysis of double-labelled cells indicated that some but not all RyR clusters colocalize with NCX labelling and at least 10% of the total NCX labelling was located at or near junctions. Our data also show that some RyR clusters are located between z-lines. These clusters are generally closely associated with longitudinal elements of the t-system (as identified by NCX and Cav3 labelling) suggesting that

these RyRs are in junctions with the longitudinal t-system. Our data provide 3D data sets for detailed modelling studies to improve our understanding of the potential role of NCX in modulating SR release.

2637-Plat β-adrenergic Modulation of the Intermolecular Signaling between a Single L-type Ca^{2+} Channel and Ryanodine Receptors in Rat Heart Cells

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β-Adrenergic receptor (β-AR) signaling upregulates cardiac contractility by enhancing Ca^{2+} entry from L-type Ca^{2+} channels (LCCs), Ca^{2+} release from ryanodine receptors (RyRs) and Ca^{2+} loading into sarcoplasmic reticulum (SR). In order to further dissect the mechanisms of β-AR modulation of RyR Ca^{2+} release at the molecular level, we utilized the loose-patch confocal imaging technique to investigate the effect of β-AR stimulation on the intermolecular coupling between a single LCC and corresponding RyRs in rat ventricular myocytes. In the presence of 20 mM Ca^{2+} and 10 μM LCC agonist FPL64176 in pipette electrodes, line-scan confocal imaging of fluo-4 fluorescence detected that LCC Ca^{2+} sparklets activated RyR Ca^{2+} sparks in a stochastic manner. β-AR agonist, isoproterenol (1 μM), increased spark amplitudes by more than 60% by increasing RyR Ca^{2+} release flux rather than release duration. The latency time constant for an LCC sparklet to activate a RyR spark was shortened from 4.3 ± 0.3 to 3.0 ± 0.2 ms under β-AR stimulation. The LCC-RyR coupling fidelity was also enhanced accordingly. Adjusting SR Ca^{2+} load back to control level, either by lowering extracellular Ca^{2+} concentration or by applying the SR Ca^{2+}-ATPase inhibitor cyclopiazonic acid, could eliminate part but not all of these changes. This result unmasked that, in addition to increased SR Ca^{2+} load, direct modification of RyR property also plays an important role in β-AR modulation of RyR Ca^{2+} release *in situ,* further clarifying the molecular mechanisms of adrenergic regulation of heart function.

2638-Plat The Ticking Speed of the Intracellular "Ca^{2+} Clock" and the Spontaneous Beating Rate of Sinoatrial Node Pacemaker Cells (SANC) are Regulated by Modulation of Sarcoplasmic Reticulum (SR) Ca^{2+} Pumping Kinetics and Protein Kinase A (PKA)-dependent Phosphorylation

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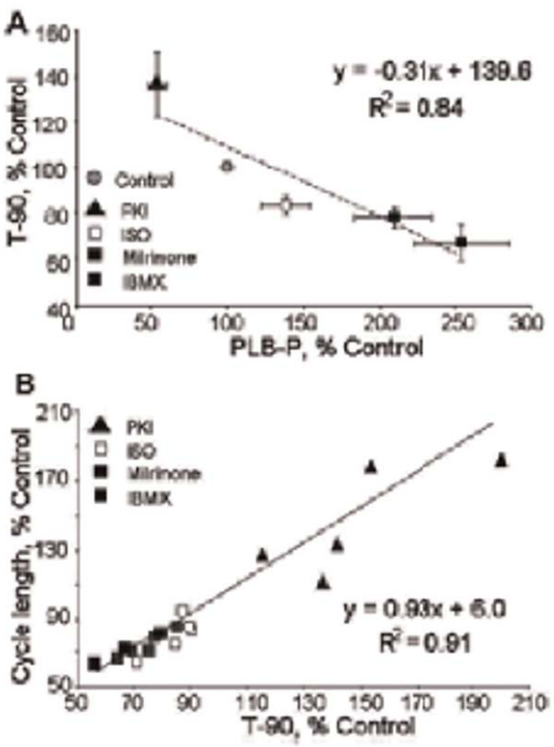
An SR "Ca^{2+} clock" within SANC generates spontaneous Local subsarcolemmal Ca^{2+} Releases (LCR) after a delay, LCR-period, following a prior AP-induced Ca^{2+} transient. The LCR period controls the spontaneous SANC cycle length, because LCR activate an inward Na^{+}-Ca^{2+} exchange current which accelerates the terminal diastolic depolarization rate. To establish links between physiological regulation of the LCR period and kinetics of SR Ca^{2+} refilling we employed, as indexes of SR pumping rate and LCR period, phosphorylation of phospholamban (PLB) and the time to 90% decay of the AP-initiated global cytosolic Ca^{2+} transient (T-90). Graded PLB phosphorylation by β-AR stimulation (ISO), a broad-spectrum phosphodiesterase inhibitor (IBMX), specific phosphodiesterase-3 inhibitor (milrinone), or by specific PKA inhibitor peptide (PKI) were paralleled by proportional changes in T-90 (Fig A). Concurrent changes in T-90 and LCR period were highly correlated with concurrent changes in the spontaneous cycle length (Fig B). Thus, cAMP-PKA-dependent modulation of the SR Ca^{2+} refilling rate defines the LCR period, "Ca^{2+} clock", and spontaneous rate of SANC.



2639-Plat Altered Cellular Ca2+ Cycling in Pharmacologically-induced LQT3 in Intact Rat Heart

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The Long QT3 Syndrome occurs as a result of genetically-induced prolongation of sodium current (INa) decay, resulting in increased action potential duration and increased intracellular Na+. We used pharmacological induction (using ATX II) of prolonged INa as a model to investigate how slowing of INa decay affected cellular Ca2

+ activity in myocytes of intact heart. Rat hearts were loaded with fluo-4AM on the stage of a confocal microscope and rapid pacing protocols were used to study intracellular Ca2+ cycling during INa prolongation. A low concentration of ATX II (1nM) was used to increase QT duration of the ECG, mimicking the LQT3 phenotype. Prolongation of INa increased the duration of basal Ca2+ transients and vulnerability to the development of cellular Ca2+ alternans induced by rapid pacing. Ca2+ waves developed during exposure to ATX II that increased in incidence and decreased in coupling interval following rapid pacing, suggesting that triggered activity was the result of Ca2+ overload and ensuing delayed afterdepolarizations (DADs). This triggered activity was probably responsible for the arrhythmias that were triggered during rapid pacing in the presence of ATX II. Our data demonstrate that the slowly inactivating INa induced by low concentrations of ATX II

1. is responsible for QT prolongation that pharmacologically mimics genetically-induced LQT3;
2. causes a prolongation of intracellular Ca2+ transients which contributes to the development of Ca2+ alternans;
3. causes intracellular Ca2+ overload and resulting Ca2+ waves, DADs and triggered activity; and
4. causes pacing-induced ventricular tachycardias by triggered activity and/or reentrant excitation.

We conclude that induction of late INa causes profound disturbances in Ca2+ cycling which may contribute to arrhythmogenesis in addition to its direct effects on INa and the cardiac action potential.

Platform BC: Protein Assemblies

2640-Plat Clathrin Triskelia Self Assemble into Fullerene Cages Permitted by the Head-to-Tail Exclusion Rule

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Originally coined for carbon cages, the word "fullerene" describes a closed, convex cage with 3-connected vertices and with hexagonal and pentagonal faces, necessarily 12 of the latter (Kroto et al., Nature *318*, 162–163, 1985). Carbon atoms self assemble into large fullerene cages that obey the isolated-pentagon rule (IPR), the smallest of which is buckminsterfullerene with n=60 vertices. Clathrin triskelia self assemble into small fullerene cages as well as large ones. The small ones have adjacent pentagons, including at least one with n=28 and two with n=36 vertices.

To explain which cages could self assemble, we proposed a "head-to-tail exclusion rule", the strong form of which permits self assembly of all IPR cages ($n>60$), buckminsterfullerene (n=60), and just 15 of the 5769 mathematically possible, small ($n\leq 60$), non-IPR cages (Schein et al., Biophy J, in press). A weak form of this rule permits 99 additional small, non-IPR cages.

Cheng et al. (JMB *365*, 892–899, 2007) recently reported four new clathrin fullerene cage structures, identified here as three that obey the strong form and one that obeys the weak form. The new tally is thus six of the 15 that obey the strong form, one of the 99 that obey the weak form, and none of the remaining 5655 that obey neither. The recent clathrin cage structures thus confirm the predictions of the head-to-tail rule and favor the strong form, though not exclusively. All carbon cages reported to date obey the strong form.

2641-Plat Protein-Protein Interfaces: Amino Acids Bias for Heterocomplexes and Homodimers

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Protein-protein interactions are involved in a variety of biological processes at the molecular level. In particular, protein-protein interactions play a crucial role in such an intriguing process as protein folding. A nonredundant set of protein-protein interfaces from PDB was used for comprehensive analysis by a rigorous nonparametric method based on Voronoy-Delaunay tessellation. Full residue contact matrixes were constructed separately for homodimers and heterocomplexes at residue and atomic levels. The distributions obtained for contact areas and distances admit interpretation in terms of the model suggesting coexistence of stochastic and specific interresidue contacts. Based on the scheme of occasionally intersecting circles, we derived formula which in principle permit one to separate the contributions from casual and specific contacts. We calculated pairing preference indexes, and found that in homodimers they are largest for contacts Cys-Cys and those between residues with opposite charges. Rather small indexes are characteristic of contacts Gly-Pro and Met-Met. A similar pattern is observed for heterocomplexes. At the same time, in comparison with homodimers, we detect a higher preference index for contacts Cys-Cys and Gly-Gly but not for Met-Met. The basic difference between heterocomplexes and homodimers consists in enrichment of contacts between same residues in the latter case, even for contacts of similar charges. It may be explained by symmetric transformations in homodimers. We identified pairs of residues responsible for specific recognition and those that come in touch by chance. The biophysical nature of interactions in both cases is discussed.

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2642-Plat Revealing Physical Basis For Ruggedness Of Protein-protein Energy Landscapes: Interaction Cutoff Effect

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The concept of the energy landscape is important for better understanding of protein-protein interactions and for designing adequate docking procedures [1,2]. The intermolecular landscape has a rugged terrain that impedes search procedures. Its inherent rugged-